EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1130

EFFECTS OF NITRAMINE EXPLOSIVE CL-20 ON THE SOIL MICROINVERTEBRATE COMMUNITY IN A SANDY LOAM SOIL

Roman G. Kuperman
Ronald T. Checkai
Carlton T. Phillips
Michael Simini
J. Steven Anthony

RESEARCH AND TECHNOLOGY DIRECTORATE

September 2013

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
XX-09-2013	Final	Jul 2002 - Aug 2012

4. TITLE AND SUBTITLE
Effects of Nitramine Explosive CL-20 on the Soil Microinvertebrate Community in a Sandy Loam Soil

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Kuperman, Roman G.; Checkai, Ronald T.; Phillips, Carlton T.; Simini, Michael; and Anthony, J. Steven

5d. PROJECT NUMBER
SERDP ER-1254

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRT-E, APG, MD 21010-5424

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1130

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Development Program (SERDP)
901 North Stuart Street, Suite 303, Arlington, VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
SERDP

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We investigated the effects of nitramine explosive, CL-20, on the soil microinvertebrate community in Sassafras sandy loam (SSL) soil using a 12 week soil microcosm assay. Freshly collected SSL soil was amended with CL-20 to prepare multiple treatment concentrations ranging from 0 (acetone control) to 10,300 mg kg^{-1}. The selected CL-20 concentration range adequately assessed the concentration–response relationships for total microarthropods and for individual microarthropod groups. Based on the number of taxonomic groups present in the individual treatments after 12 weeks, the overall composition of microarthropod community in SSL soil was not affected by exposure to CL-20. However, community structure analysis revealed greater sensitivity to CL-20 by predatory mesostigmatid mites. Microarthropod and nematode communities showed contrasting sensitivities to CL-20 in SSL soil. Total numbers of nematodes were unaffected or significantly ($p < 0.05$) increased in the CL-20-treated soil compared with the control. Only the predator group among nematodes was consistently adversely affected by exposure to CL-20. The abundance of predatory nematodes decreased in a concentration-dependent manner throughout the 12 week exposure. Microcosm assays with corresponding community structure analysis can be used to validate the ecotoxicity data from standardized laboratory tests and complement and expand upon the ecotoxicological significance of data from standardized single-species toxicity tests.

15. SUBJECT TERMS
Soil invertebrate community CL-20 Toxicity assessment Natural soils Ecological risk assessment

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Renu Rastogi
U	U	U	UU	34	**19b. TELEPHONE NUMBER** *(include area code)* (410) 436-7545

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PREFACE

The work described in this report was authorized under Project No. SERDP ER-1254. The work was started in July 2002 and completed in August 2012.

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This report has been approved for public release.

Acknowledgments

The authors thank Geoffrey I. Sunahara and Jalal Hawari from Biotechnology Research Institute, National Research Council Canada for their contributions of methodology development, interlaboratory participation in quality control assurance measures for analytical determinations of CL-20, and data exchange during execution of this project. This project was completed in cooperation with and funding by the Strategic Environmental Research and Development Program (SERDP), Arlington, VA.

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1. INTRODUCTION

Preservation of soil fertility and structure is essential to protect and sustain the ecological integrity of terrestrial ecosystems at military installations. Important to achieving this goal is understanding the potential impacts on the soil ecosystems of an accidental release of explosives during manufacture or use in training, storage, or disposal operations. Soil contaminated with energetic materials can affect soil biota directly or indirectly by altering specific interactions among the populations of soil organisms and by disrupting soil food webs. Populations of soil organisms are intimately linked, and the effects of chemicals on any one species or group can impact the whole community. Ultimately, these effects can interfere with key soil processes that are important to the regulation, flow, and internal cycling of carbon and nutrients in ecosystems (Edwards and Bohlen, 1995; Kuperman and Carreiro, 1997; Kuperman et al., 1998; Parmelee et al., 1993). The use of multispecies tests while assessing soil contamination offers holistic tools for risk assessment and can provide a much broader understanding of the mechanisms by which soil contamination can affect the structure and function of soil ecosystems (Kuperman et al., 2002).

In spite of advances in soil ecotoxicological methodologies (Kuperman et al., 2002, 2009), only a few studies have investigated the community-level effects of soil contamination (Bogomolov et al., 1996; Parmelee et al., 1993, 1997; Kuperman et al., 2007; Scott-Fordsmand et al., 2008). Soil microcosms can be used as tools to assess the community-level effects of chemicals and provide a large set of measurement endpoints from which an appropriate group can be selected for specific ecosystem structures and functions (Kuperman et al., 2002; Wentsel et al., 2003). Currently, the limited data derived from standardized single-species laboratory tests are used to assess ecological risk in terrestrial ecosystems, and these findings are extrapolated to contaminated sites without sufficient comprehensive regard to complexity of soil ecosystems in the field. Toxicity data established in standardized single-species tests can under- or overestimate the potential exposure effects on soil invertebrates in the field. For example, in a 7 day microcosm assay, total microarthropod numbers were reduced by 50% in the 30 mg kg^{-1} 2,4,6-trinitrotoluene (TNT) treatment compared with numbers in control oak–beech forest silt loam soil (Parmelee et al., 1993). This reduction suggested an order of magnitude greater toxicity when contrasted with the median lethal concentration for 50% of the population (LC_{50}) value of 360 mg kg^{-1} reported for acute effect (14 days) of TNT on adult enchytraeid worm (potworm) *Enchytraeus crypticus* in freshly amended Sassafras sandy loam (SSL) soil (Kuperman et al., 2005).

Evaluation of the ecotoxicological data established for an emerging polynitramine energetic material, 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (China Lake compound 20 [CL-20]), in standardized single-species tests indicated very high toxicity to soil invertebrates (Dodard et al., 2005; Kuperman et al., 2006a, 2006b; Robidoux et al., 2004). However, such ecotoxicological data did not account for the community-level effects of exposure to test chemicals or for possible interactions among the soil invertebrate populations. To address the knowledge gap regarding the soil invertebrate community-level effects of CL-20,

we designed our studies to test the hypotheses that the effects of CL-20 on the indigenous soil microinvertebrate community will be specific for individual taxonomic or trophic groups and will be influenced by the duration of chemical exposure for these organisms in a natural soil. These tests were accomplished by conducting definitive microcosm toxicity assays with soil containing the indigenous microinvertebrate community. The objectives of this investigation included (i) assessing the respective toxicities of CL-20 to the soil microinvertebrate community groups, (ii) determining whether the toxicities of CL-20 to individual groups within the soil microinvertebrate community can be affected by the duration of exposure, and (iii) assessing the utility of the microcosm assay as a tool for developing ecotoxicological parameters for use in the ecological risk assessment of contaminated soils.

2. MATERIALS AND METHODS

2.1 Soil Collection and Preparation

A natural soil, SSL (fine-loamy, siliceous, semiactive, mesic Typic Hapludult; U.S. Department of Agriculture, Natural Resources Conservation Service, Agricultural Research Service [USDA-NRCS//ARS], 1999), was used in this investigation to assess CL-20 toxicity to the soil microinvertebrate community. This soil was selected for developing ecotoxicological values protective of soil biota because (i) it was previously used to establish ecotoxicological benchmarks for CL-20 in standardized soil invertebrate toxicity tests (Dodard et al., 2005; Kuperman et al., 2006a, 2006b), and (ii) it has physical and chemical characteristics that support "very high" qualitative relative bioavailability for organic chemicals in natural soils (U.S. Environmental Protection Agency [USEPA], 2005). These characteristics include low organic matter (OM) and clay contents (2.6% OM, 14% clay, 58% sand, and 28% silt), 9.8 cmol kg^{-1} cation-exchange capacity, and pH 5.1. Total concentrations of metals and nutrients were within regional background ranges and were reported previously (Robidoux et al., 2004).

In May 2003, fresh SSL soil containing the indigenous microinvertebrate community was collected from an open grassland field in the coastal plain on the property of the U.S. Army Aberdeen Proving Ground, Harford County, MD. Soil was gently sieved using a 5 mm sieve to remove large debris and regularize distribution of soil invertebrates. Samples (n = 5) of prepared soil were extracted immediately to establish the baseline data for abundance of microarthropods and nematodes in SSL soil. This baseline data was used to determine the effects of subsequent soil preparation procedures on the soil microinvertebrate community. Prepared soil was stored in covered plastic containers overnight to preserve the initial field moisture content. Dry SSL soil that was collected earlier and sieved through a 2 mm sieve was used to prepare the CL-20 soil concentrates. Soil concentrates were required to uniformly amend the fresh field-moist SSL soil during preparation of nominal target treatment concentrations without harming soil organisms by exposure to solvent. During the soil concentrate preparation procedure, appropriate amounts of CL-20 were amended into separate aliquots of soil using an organic solvent (acetone) as a carrier. This was necessary to more evenly and uniformly distribute the CL-20 to a large soil surface area, rather than add solid chemical crystals to soil. Soil was spread to a thickness of 2.5 cm. CL-20 was dissolved in acetone in glass volumetric flasks then pipetted across the soil surface, while ensuring that the volume of solution added at

any one time did not exceed 15% (v/w) of the soil dry mass. After the designated CL-20 solution was added to a respective aliquot of SSL soil, the volumetric flask was rinsed twice with a known volume of acetone and these rinses were also pipetted onto the soil. If the total volume of solution needed to amend the soil exceeded 15% (v/w), the solution was added in successive stages to allow the acetone to evaporate between additions for a minimum of 2 h in darkness within a chemical hood. The same total CL-20/acetone solution volume, at different CL-20 concentrations, was added to every treatment, which equaled the volume required to dissolve CL-20 at the greatest dissolved concentration amended. This approach was used to prepare nominal CL-20 treatments of 100, 500, 1000, and 2500 mg kg^{-1}. The nominal CL-20 treatments of 5000; 7500; and 10,000 mg kg^{-1} substantially exceeded the solubility levels of CL-20 in an acetone carrier, so these were prepared by directly mixing the appropriate amounts of dry crystalline CL-20 with dry SSL soil. Acetone was added to these three treatments in the same amount that was used in the preparation of other treatments to maintain the uniformity of treatments (i.e., solvent addition) throughout all exposure concentrations. Amended soil was subsequently air-dried overnight (minimum of 18 h) in a darkened chemical hood to prevent photolysis of CL-20. Each soil treatment sample was then transferred into a fluorocarbon-coated, high-density polyethylene container and mixed for 18 h on a three-dimensional rotary soil mixer.

One day after collecting soil in the field, nominal target concentrations for all freshly amended treatments were prepared by individually combining and gently mixing CL-20-amended soil concentrates with clean SSL field soil in a plastic bag. This approach ensured that the amount of fresh SSL soil containing indigenous soil biota remained constant throughout the range of treatments. The carrier control treatment was amended with acetone-treated SSL soil only. The field soil moisture level, which was determined at the time of soil collection, was maintained for the duration of the studies by adding reagent water to maintain the appropriate total weight. The final nominal CL-20 treatment concentrations prepared for definitive testing included 0 (carrier control); 100; 500; 1000; 2500; 5000; 7500; and 10,000 mg kg^{-1}. Soil sample extracts from each treatment were analyzed using high-performance liquid chromatography (HPLC) with a modified USEPA Method 8330A (USEPA, 2007) to determine acetonitrile-extractable CL-20 concentrations. Exposure concentrations of CL-20, which were determined analytically at commencement of the definitive test, were below detection limit (BDL) in carrier control; 108; 479; 870; 2355; 4635; 7020; and 10,300 mg kg^{-1}. Concentrations of CL-20 in all treatments at each harvest date are shown in Table 1.

2.2 Chemicals and Reagents

Crystalline CL-20 (Chemical Abstracts Service [CAS] no. 135285-90-4; ε-isomer, purity 99.3%) was obtained from ATK Thiokol Propulsion (Ogden, UT). HPLC-grade acetone (CAS no. 67-64-1; Fisher Scientific; Pittsburgh, PA) was used to prepare individual CL-20 solutions prior to soil amendments. Acetonitrile (CAS no. 75-05-8; HPLC grade; Pharmco; Brookfield, CT), methanol (CAS no. 67-56-1; chromatography grade; purity 99.9%; Pharmco), and calcium chloride ($CaCl_2$; CAS no. 10043-52-4; reagent grade; purity 100%; J.T. Baker; Phillipsburg, NJ) were used for the soil extractions and for analytical determinations using HPLC. Ethanol (CAS no. 64-17-5; purity 99.98%; Pharmco) was used as a preservative for the extracted microarthropods. Sodium bisulfate monohydrate ($NaHSO_4 \cdot H_2O$; CAS no. 10034-88-5; purity 99%; Sigma-Aldrich; St. Louis, MO) was used to acidify stock solutions in preparation of

chemical extracts from soil for determinations using HPLC. American Society for Testing and Materials (ASTM) type I reagent water (18 MΩ cm at 25 °C; ASTM, 2004) was used throughout the toxicity studies. It was obtained using the Milli-RO 10 Plus followed by the Milli-Q PF Plus systems (Millipore; Bedford, MA). The same grade of reagent water was used throughout the analytical determinations. Glassware was washed with phosphate-free detergent, followed by rinses with tap water, ASTM type II water (>5 MΩ cm at 25 °C), analytical reagent grade nitric acid 1% (v/v), and then with ASTM type I water.

2.3 Chemical Extractions and Analyses

For all control and treated soils, concentrations of CL-20 were analytically determined in triplicate, at the beginning of the definitive test and after 4, 8, and 12 weeks, using acetonitrile extraction and USEPA Method 8330A (USEPA, 2007). From each treatment soil batch, 2.3 g of soil was weighed in triplicate replication into a 50 mL polypropylene centrifuge tube, 10 mL of acetonitrile was added, and the samples were vortexed for 1 min then sonicated in darkness for 18 h at 20 °C. Sonicated samples were centrifuged at 2700 rpm for 30 min. Five milliliters of the resulting supernatant were transferred to a 20 mL glass vial and combined with 5 mL of $CaCl_2$/$NaHSO_4$ aqueous solution (5 and 0.2 g L^{-1}, respectively), which was used as a flocculent. The samples were shaken and left to equilibrate and settle for 30 min. The supernatant was filtered using disposable syringes (10 mL) and 0.45 μm Millipore polytetrafluoroethylene (PTFE) syringe filters. The first 3 mL of filtrate was discarded, and the remainder was retained in a PTFE-capped 4 mL vial. One milliliter of this filtered solution was transferred to a HPLC vial. The filtered samples were stored in the refrigerator at 4 °C in darkness; the samples were stored no longer than 5 days, if they were not analyzed on the same day. Soil extracts were analyzed and quantified using a modified EPA Method 8330A (USEPA, 2007). The results of acetonitrile soil extractions are reported as CL-20 concentrations in oven-dry soil.

Concentrations of CL-20 in the soil extracts were determined using the HPLC–UV system, which consisted of an Agilent 1100 HPLC Series (Aglient Technologies; Santa Clara, CA) equipped with a Supelcosil LC–cyano (CN) column (25 cm × 4.6 mm × 5 μm), and employed an isocratic 70:30 methanol:water mobile phase with a flow rate of 1.0 mL min^{-1} and a 50 μL injection volume. The autosampler temperature was set to 10 °C. Blanks and standards were placed among samples having unknown concentration to maintain quality assurance of the samples. Detection of CL-20 was accomplished using a diode array detector at a 230 nm wavelength. A primary stock solution was prepared at 10,000 mg L^{-1} of CL-20 in acetonitrile. Intermediate stock solutions of 50, 20, 2, 0.5, and 0.1 mg L^{-1} of CL-20 in acetonitrile were then prepared from the primary stock solution. Calibration standards were made from the intermediate stock solutions with acidified water (sodium bisulfate) solution (50:50) to yield standards of 25, 10, 1, 0.25, and 0.05 mg L^{-1} of CL-20 in acetonitrile/H_3O^+. Calibration curves were created (r^2 > 0.99999) with an instrument limit of detection (LOD) of 0.01 mg L^{-1} (signal-to-noise ratio = 3). Over 5 months, the reproducibility of the slope was determined to be 149.0 ± 5.0 with a percent relative standard deviation (%RSD) of 3.4 (n = 14). All exposure treatments used in the microcosm assay were above the method detection limit of 0.06 mg kg^{-1} for soil CL-20 concentrations.

2.4 Experimental Approach

The duration of the definitive test was 12 weeks and included three harvests at 4 week intervals to allow for assessment of the potential effects of weathering-and-aging of CL-20 in soil on the resulting CL-20 bioavailability and toxicity to soil biota. The definitive test was conducted using four replicates per treatment per harvest date (total of 12 test containers per treatment). Approximately 200 g of treated soil was loosely packed into individual test containers (900 mL, 90 mm diameter glass jars). The total mass of each test container with soil was recorded. Plastic wrap was stretched over the top of each container and secured with a rubber band. Three pinholes were made in the plastic wrap to facilitate air exchange. All containers were randomly placed in an environment-controlled incubator under a 16 h light, 8 h dark photoperiod cycle, with a mean photosynthetically active radiation (PAR) light intensity of 12.8 ± 0.7 (standard error) µM m^{-2} sec^{-1} (985 ± 52 lux), a mean temperature of 22 ± 1 °C, and 86% relative humidity (RH). ASTM type I water was added every week to maintain the initial soil moisture level.

A set of four randomly selected replicates from within each treatment was processed during the first, second, and third (final) harvests, after the 4, 8, and 12 week exposure periods, respectively. Soil was gently mixed inside individual test containers and then sampled to determine percent moisture and CL-20 concentrations in test soil and to extract microarthropods and nematodes. Soil microarthropods were extracted into 76% ethyl alcohol using high-gradient extractor (Merchant and Crossley, 1970). The most abundant microarthropods were sorted to acarine suborders Prostigmata, Mesostigmata, and Oribatida, and the hexapod subclass Collembola (springtails). Other arthropods including the classes Diplopoda and Symphyla and the hexapod orders Diplura, Psocoptera (psocids), Thysanóptera (thrips), Coleoptera (beetles) larvae, and Diptera (flies) larvae were recorded and added to the total number for statistical analyses. Nematodes were extracted using the Baermann funnel method (McSorley and Walter, 1991) for 48 h at room temperature (Parmelee et al., 1993). Live nematodes were counted at 140× visual magnification and were sorted into bacterivore, herbivore, fungivore, omnivore, and predator trophic groups, and into a group of hatchlings as described in Parmelee et al. (1993). The abundance of soil invertebrates was expressed as number of individuals per gram of dry soil (ind g^{-1}).

2.5 Data Analyses

Soil microinvertebrate data were analyzed using regression models selected from among those described in the guidance document EPS 1/RM/46 from Environment Canada (2005) and Stephenson et al. (2000). Survival data was normalized using square root ($x + 1$) transformation prior to regression analyses; the exception was predatory nematode survival data from the second harvest that did not require transformation. Histograms of the residuals, stem-and-leaf graphs, and variances of the residuals were examined to ensure that normality assumptions were met and to decide whether to weight the data. This information was also used in selecting the appropriate models.

Exponential model, $Y = a \times e^{(\{[\log(1-p)] / EC\mathrm{p}\} \times C) + b}$, had the best fit for survival data for individual groups of soil microinvertebrates; where Y is the dependent variable for a

measurement endpoint (e.g., number of oribatid mites); *a* is the *y*-axis intercept (i.e., the control response); *e* is the exponent of the base of the natural logarithm; *p* is the desired value for *p* effect (e.g., 0.50 for a 50% decrease from the control response; effective concentration producing a 50% decrease in measurement endpoint [EC_{50}]); *C* is the exposure concentration in test soil; *EC*p is the estimate of effective concentration for a specified percent effect; and *b* is a scale parameter that defines the shape of the equation. The best fit of the curves generated by these models were closest to the data points, the variances of the residuals were the smallest, and the residuals had the best appearance (i.e., most-random scattering). The *EC*p parameters used in these studies included the effective CL-20 concentration producing a 20% (EC_{20}) or 50% (EC_{50}) reduction in the abundance of affected soil microinvertebrate group compared with carrier control. The 95% confidence intervals (CIs) and regression coefficients (r^2) were determined for all *EC*p endpoint estimates.

Analysis of variance with untransformed data was used to determine the bounded (when possible) no-observed-effect concentration (NOEC) and lowest-observed-effect concentration values at the $p \leq 0.05$ level for the abundance of soil microinvertebrate groups compared with the carrier control. Mean separations were done using Fisher's least-significant difference (FLSD) pairwise comparison tests. The Student's *t*-test (two-tailed) was used to analyze the baseline and control data for microinvertebrate groups. All analyses were done using measured CL-20 concentrations. Statistical analyses were performed using SYSTAT 11 (Systat Software, Inc.; Chicago, IL).

3. RESULTS AND DISCUSSION

Results of analytical determination showed that the initial concentrations of CL-20 averaged 96 ± 3% of nominal concentrations, which confirmed the precision of the treatment preparation procedures (Table 1). Concentrations of CL-20 in SSL soil treatments remained relatively stable during the 12 week microcosm studies, averaging 93 ± 2, 96 ± 4, and 97 ± 4% of the initial concentration in freshly amended soil after 4, 8, and 12 weeks, respectively (Table 1).

The total abundance of microarthropods was not affected (Student's *t*-test, $p = 0.490$) by the soil preparation procedures. This conclusion was based on comparison of the baseline data (1.1 ± 0.1 ind g^{-1}, $n = 5$) and the data from control samples extracted after the first harvest (0.96 ± 0.2 ind g^{-1}, $n = 4$). The abundance of nematodes in similarly replicated samples was significantly greater (Student's *t*-test, $p = 0.025$) in the control (34.3 ± 4.5 ind g^{-1}) after the first harvest compared with the baseline data (17.2 ± 1.8 ind g^{-1}). The overall numbers of microarthropods and nematodes increased in the control treatments by 242 and 423%, respectively, by the end of the 12 week study. These results confirmed that soil preparation procedures and the controlled abiotic environmental conditions in test containers (soil moisture, temperature, RH, photoperiod, etc.) did not adversely affect the soil microinvertebrate community in SSL soil during assessment of the effects of exposure to CL-20.

Table 1. Concentrations of CL-20 in SSL Used in the 12 Week Microcosm Studies with Indigenous Soil Microinvertebrate Community

Nominal (mg kg^{-1})	Initial Determined (mg kg^{-1})		Harvest I (mg kg^{-1})		Harvest II (mg kg^{-1})		Harvest III (mg kg^{-1})	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
100	108	4	105	6	90	5	91	4
500	479	18	480	19	385	32	485	13
1000	870	14	836	36	843	33	1021	23
2500	2355	61	2211	132	2318	141	2392	136
5000	4635	235	4068	709	5236	472	4251	227
7500	7020	268	6345	218	7032	218	6214	442
10,000	10,300	303	8689	727	10,011	291	9615	844

Notes: Replicate microcosm units were harvested at 4 week intervals. Analytically determined concentrations (means and standard errors [SE]; $n = 3$) were based on acetonitrile extraction and determinations using HPLC with USEPA method 8330A. Method detection limit was 0.06 mg kg^{-1}.

Exposure to CL-20 had an immediate adverse effect on the microarthropod community (Figures 1 and 2). The total numbers of microarthropods were significantly ($p = 0.007$) decreased in the first concentration tested (105 mg kg^{-1}) and remained significantly ($p < 0.0001$) lower when compared with the control in the greater CL-20 treatments after 4 weeks of exposure. After 8 weeks of exposure, total numbers of microarthropods were significantly ($p \leq 0.033$) lower in the ≥5236 mg kg^{-1} treatments. By the end of the 12 week exposure to CL-20, the total abundance of microarthropods was not significantly ($p \geq 0.123$) different from the control in any of CL-20 treatments, although the total numbers continued to decline in a concentration-dependent manner.

The selected concentration range of CL-20 was used to adequately assess the concentration–response relationships for total microarthropods in SSL soils (Figure 2). Similar relationships were established for individual microarthropod groups (graphics are not shown) and allowed us to determine group-specific ecotoxicological benchmarks for CL-20. High variability in the abundance of microarthropods, which is common for natural soil ecosystems, produced relatively wide ranges of the 95% CI for endpoint estimates for all taxa except mesostigmatid mites (Table 2). This predatory group of mites had the lowest estimated EC_{50} values on the first and third harvests, but mesostigmatid endpoints were preceded by corresponding oribatid mites on the second harvest. The final order of EC_{50} values (from lowest to greatest) after 12 weeks was Mesostigmata < Collembola < Prostigmata < Oribatida (Table 2).

Based on the number of taxonomic groups present in the individual treatments after 12 weeks (Figure 1), the overall composition of microarthropod community in SSL soil was not affected by exposure to CL-20. Community structure analysis revealed greater sensitivity to CL-20 of predatory mesostigmatid mites, which were absent in the 6345 mg kg^{-1} treatment after 4 weeks and in the 843, 2318, 7032, and 10,011 mg kg^{-1} treatments after 8 weeks of exposure to CL-20 (Figure 3). By the end of the 12 week exposure, mesostigmatid mites were present in all CL-20 treatments (Figures 1 and 3) although their relative abundances (expressed as percent of

total abundance) remained lower when compared with the control (Figure 3). The order of relative abundance of microarthropods (from greatest to least) was Prostigmata > Oribatida ≥ Collembola > Mesostigmata (Table 2, Figure 1). Soil arthropods belonging to classes Diplopoda and Symphyla and hexapod orders Diplura, Psocoptera, Thysanóptera, Coleoptera, and Diptera were found among most CL-20 treatments after 4 and 8 weeks of exposure but disappeared from all treatments (including the control) after 12 weeks.

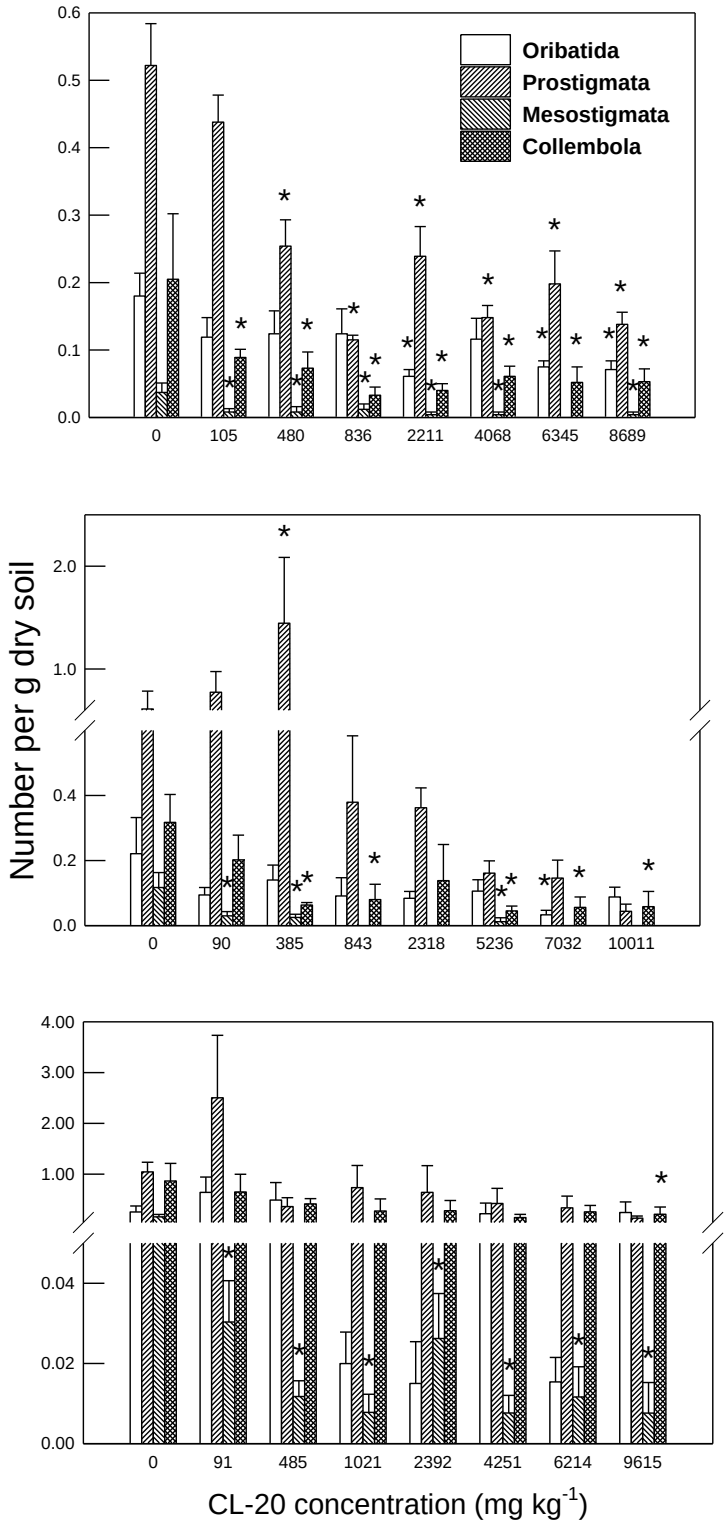


Figure 1. Abundance of microarthropod groups after (top) 4, (middle) 8, and (bottom) 12 weeks of exposure to CL-20 in SSL soil. Significant ($p \leq 0.05$, FLSD test) change from control is indicated by [*].

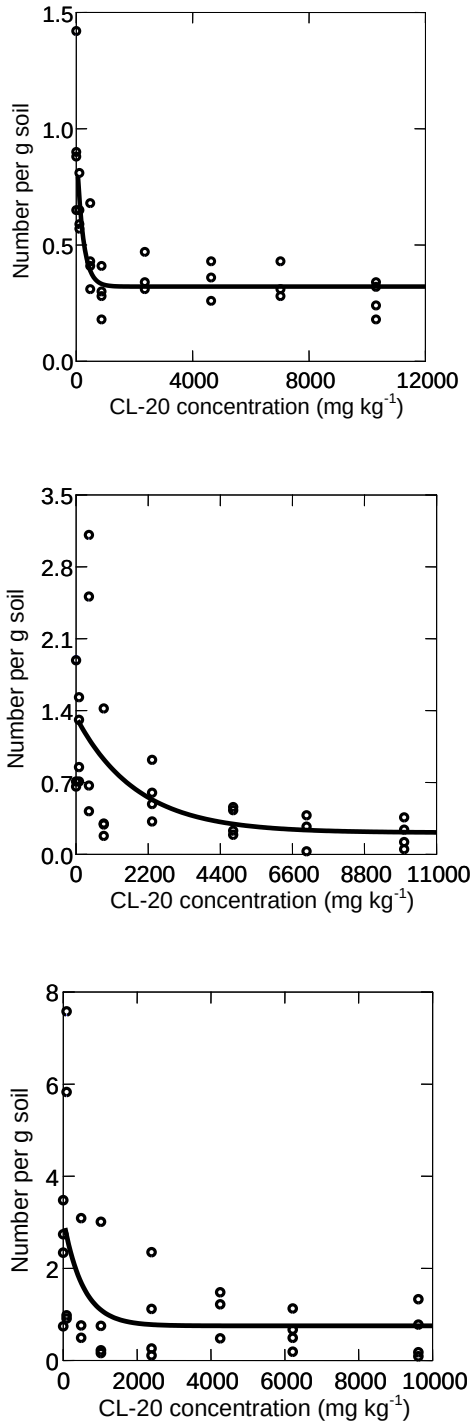


Figure 2. Effect of CL-20 on total abundance of microarthropods after (top) 4, (middle) 8, and (bottom) 12 weeks of exposure to CL-20 in SSL soil. Concentration–response relationships were established using analytically determined CL-20 concentrations and exponential model $Y = a \times e^{(\{[\log(1-p)] / EC\mathrm{p}\} \times C) + b}$.

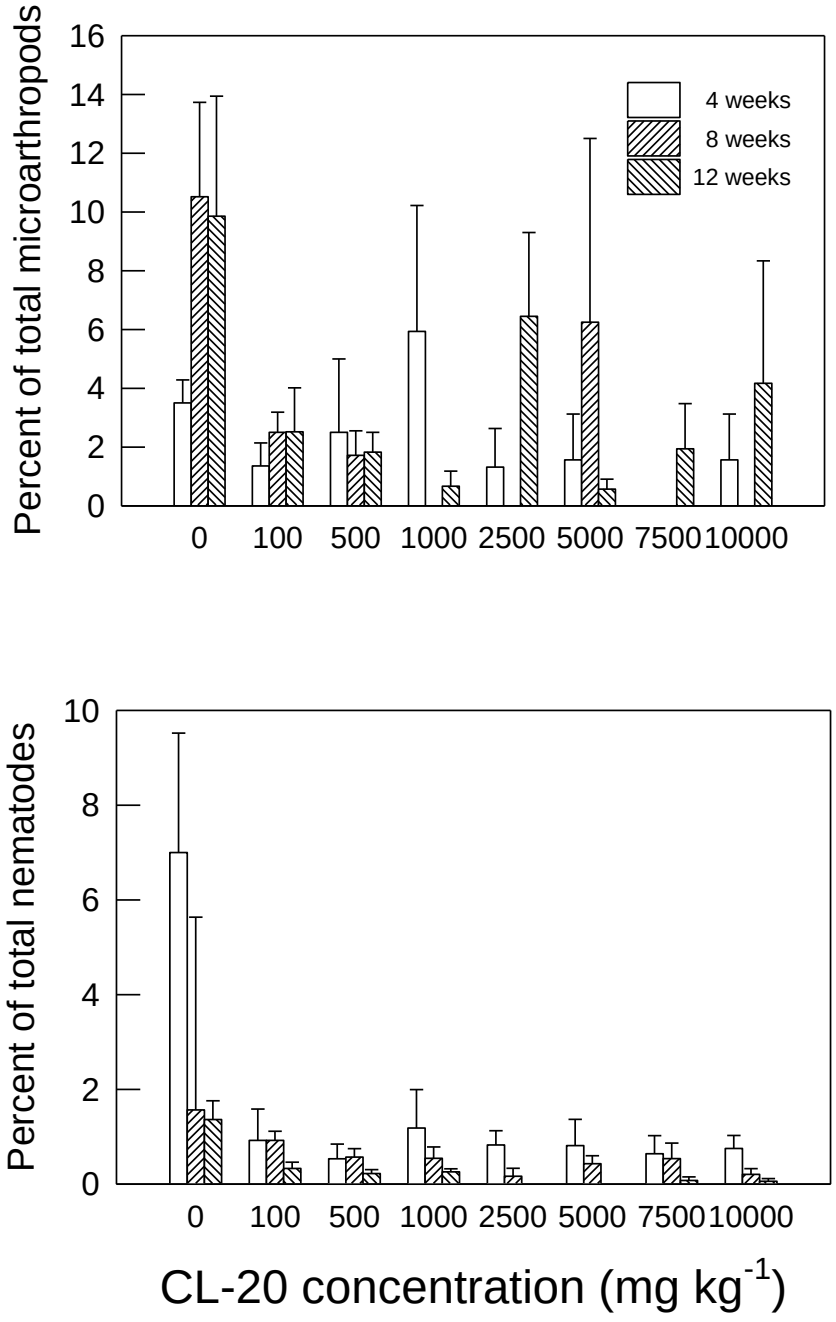
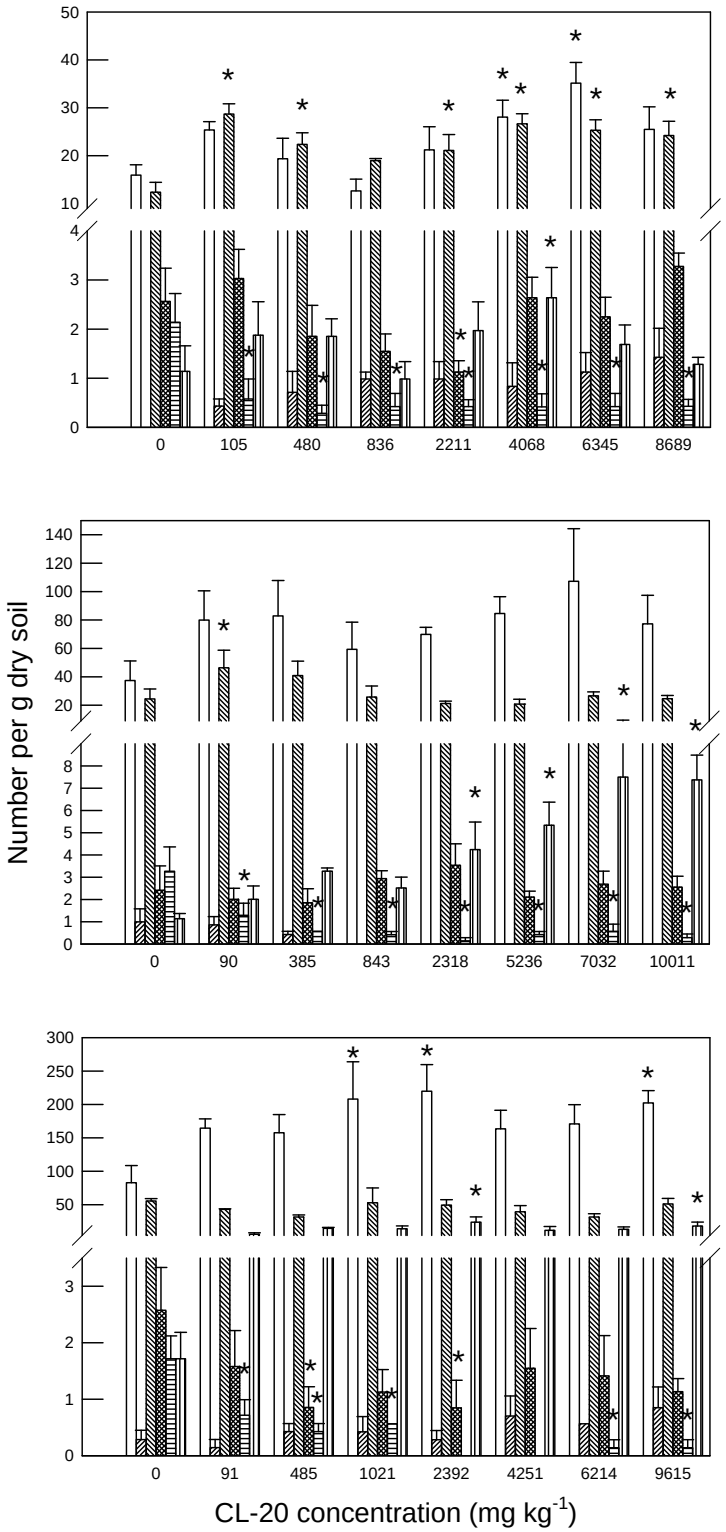


Figure 3. Relative abundance of (top) predatory mesostigmatid mites and (bottom) predatory nematodes in SSL soil amended with CL-20. Values are means and SE (n = 4). Nominal CL-20 concentrations are shown. Analytically determined concentrations for each exposure period are reported in Table 1.

Indigenous microarthropod and nematode communities showed contrasting sensitivities to CL-20 in SSL soil. The total numbers of nematodes were either unaffected ($p > 0.05$) or significantly ($p < 0.05$) increased in the CL-20 treatments when compared with the control (Figure 4). Relative abundances of trophic groups of nematodes varied among the harvest dates (Figure 4). After the 8 week exposure to CL-20, bacterivore and fungivore nematodes equally dominated (44 ± 2%) the nematode community followed by omnivore (4.6 ± 0.5%), hatchling (3.3 ± 0.3%), herbivore (1.6 ± 0.3%), and predator (1.6 ± 0.8%) groups. The relative abundance of bacterivore nematodes increased over time to 66 ± 3 and 74 ± 2% after 8 and 12 weeks, respectively. Similarly, relative abundance of the hatchling group increased over time to 3.7 ± 0.6 and 5.2 ± 0.8% after 8 and 12 weeks, respectively. Correspondingly, after 8 and 12 weeks, the relative abundances of fungivore, omnivore, herbivore, and predator groups decreased from 26 ± 2.5 to 20 ± 2.7%, from 2.4 ± 0.3 to 0.7 ± 0.2%, from 0.3 ± 0.2 to 0.2 ± 0.03%, and from 0.6 ± 0.2 to 0.3 ± 0.2%, respectively.

Among the nematodes, only the predator group was consistently adversely affected by exposure to CL-20 (Figure 3, bottom). The abundance of predatory nematodes decreased in a concentration-dependent manner throughout the 12 week exposure, producing the EC_{50} values of 31, 55, and 68 mg kg^{-1} after 4, 8, and 12 weeks, respectively (Table 2). Similarly, a greater sensitivity of the predatory nematodes to chemical exposures, compared with the other trophic groups of the nematode community, was observed in studies with copper and *p*-nitrophenol by Parmelee et al. (1993). Intermittent significant ($p \leq 0.05$) decreases, compared with the control treatment, were found for omnivore nematodes in the 2211 mg kg^{-1} treatment after 4 weeks, and in the 485 and 2392 mg kg^{-1} treatments after 12 weeks. The abundance of herbivore nematodes was significantly ($p = 0.01$) lower in the ≥843 mg kg^{-1} treatments compared with the control after 8 weeks. When identifying the effects of CL-20 on the soil microinvertebrate community, detection of these group-specific responses demonstrated the advantage of using trophic structure analysis compared with reliance only on the total abundance numbers.

The contrasting effects of exposure to CL-20 on nematodes and soil microarthropods can be related to differences in the chemical environments of the soil microsites inhabited by each of these organisms (e.g., the water film around soil particles and air-filled soil pores). These differences demonstrated that chemical bioavailability can affect the exposure and resulting toxicity of CL-20 to the two distinct groups of the soil invertebrate community. The exposure of nematodes to sparingly water-soluble CL-20 (3.7 mg L^{-1} at 25 °C; Monteil-Rivera et al., 2004) could be relatively decreased because nematodes occupy soil-pore water and water films surrounding soil particles. This decrease in exposure has likely contributed to the significantly lower toxicity of CL-20 for nematodes compared with microarthropods, which occupy soil surfaces in air-filled soil pores and therefore received greater exposure to CL-20, especially when the soil contained high concentrations of CL-20.



Legend: Bacterivore, Herbivore, Fungivore, Omnivore, Predator, and Hatchling.

Figure 4. Abundance of trophic groups of nematodes after (top) 4, (middle) 8, and (bottom) 12 weeks of exposure to CL-20 in SSL soil. Significant ($p \leq 0.05$, FLSD test) change from carrier control is indicated by [*].

Table 2. Toxicity Benchmarks and Relative Abundance of Soil Microinvertebrates Established in Microcosm Studies after 4, 8, and 12 Weeks of Exposure in CL-20-Amended SSL Soil

Invertebrate Group	4 Weeks				8 Weeks				12 Weeks			
	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})	r^2	Percent of Total*	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})	r^2	Percent of Total*	EC_{20} (mg kg^{-1})	EC_{50} (mg kg^{-1})	r^2	Percent of Total*
Oribatida	166	516	0.999	26 (3)	6	19	0.998	20 (5)	185	575	0.984	16 (6)
95% CI	0–466	0–1446			0–48	0–149			0–601	0–1867		
Prostigmata	63	197	0.999	55 (3)	691	2145	0.979	59 (7)	88	274	0.942	47 (7)
95% CI	20–107	62–332			0–2182	0–6777			0–319	0–991		
Mesostigmata	10	31	0.999	2.2 (0.6)	14	42	0.999	2.6 (1.4)	10	31	0.999	3.5 (1.1)
95% CI	0–30	0–95			0.2–27	0.6–84			0.4–20	1.3–61		
Collembola	18	55	0.999	16 (1)	29	90	0.998	19 (3)	47	146	0.975	28 (4)
95% CI	0–43	0–132			0–72	0–225			0–218	0–677		
Total microarthropods	54	168	0.998	100	428	1329	0.980	100	96	300	0.932	100
95% CI	16–92	50–285			0–1068	0–3317			0–299	0–928		
Predatory nematodes	10	31	0.977	1.6 (0.8)	18	55	0.719	0.6 (0.2)	22	68	0.985	0.3 (0.2)
95% CI	0–27	0–85			2–34	5–104			2–42	7–130		

Notes: Numbers representing relative abundances of individual groups are mean percent (and SE, $n = 8$) of the total (not reported in this table) microarthropod or nematode numbers in all treatments for each exposure period. Concentrations are based on acetonitrile extraction and HPLC using USEPA method 8330A. Method detection limit was 0.06 mg kg^{-1}.

While assessing CL-20 toxicity to the soil invertebrate community, special consideration was given to the effects of weathering-and-aging of CL-20 in soil on the exposure of soil receptors. The duration of microcosm assay and chronosequential harvesting of subset replicate units at 4 week intervals enabled the CL-20 toxicity assessment to include (1) the acute effects of freshly amended CL-20, which approximated exposure conditions in standardized single-species tests, and (2) the chronic effects of prolonged exposure, which integrated the weathering-and-aging of CL-20 in SSL soil. Weathering-and-aging of CL-20 in SSL soil more closely approximated the exposure effects in field locations where CL-20 may persist for extended periods of time. The changing exposure conditions during the 12 week studies included potential alteration of CL-20 bioavailability in soil for microinvertebrates. This was evident from the trends in toxicity data (not statistically significant on the 95% CI basis) for selected groups of microarthropods and nematodes. The EC_{50} for Collembola steadily increased (i.e., decreasing toxicity) with values of 55, 90, and 146 mg kg^{-1} after 4, 8, and 12 weeks of exposure, respectively. Corresponding values for prostigmatid mites increased yielding 197, 2145, and 274 mg kg^{-1} after 4, 8, and 12 weeks of exposure, respectively. A similar increase in EC_{50} values (from 31 to 68 mg kg^{-1}) was established for predatory nematodes. In contrast, the toxicity of CL-20 to oribatid mites increased after 8 weeks (i.e., lower EC_{50} value) compared with toxicity after 4 weeks, but later returned to approximately initial level (Table 2).

Evaluation of these results showed

- the complexity of possible interactions among the fate processes of weathering-and-aging of CL-20 in soil;
- the changes in bioavailability of the parent material and the possible degradation products of CL-20 (Balakrishnan et al., 2003, 2004a, 2004b; Trott et al., 2003); and
- the effects on different taxonomic groups that represent a range of sensitivities, which often correlate with physiologically determined mechanisms of toxicity that vary among taxa.

Including weathering-and-aging components in the CL-20 toxicity assessments allowed us to incorporate potential alterations in the soil chemical environment and corresponding changes in toxicity at contaminated sites into the development of toxicological benchmarks for the soil invertebrate community.

Overall toxicity data for soil microarthropods and predatory nematodes established in this microcosm assay were comparable with the mortality data established in our standardized single-species toxicity tests (Kuperman et al., 2006a, 2006b) with soil invertebrates using the earthworm *Eisenia fetida* (ISO,1998a), potworm *E. crypticus* (ISO, 2004), and collembolan *Folsomia candida* (ISO,1998b). These species were exposed to CL-20 in a similar SSL soil, and the results are listed in Table 3.

Results from the present studies also comport generally with mortality data from the available published studies of CL-20 effects on the earthworm *Eisenia andrei* (Robidoux et al., 2004) and enchytraeids (Dodard et al., 2005; Kuperman et al., 2006a). Robidoux et al. (2004) reported an LC_{50} value of 53.4 mg kg^{-1} (46–63 mg kg^{-1}, 95% CI) for

adult *E. andrei* in a similar SSL soil and the unbounded NOEC value of 125 mg kg^{-1} in a forest soil formulation (41% OM, pH 8.2), following a 4 week exposure to CL-20 in freshly amended soils. Dodard et al. (2005) established lower LC_{50} values that ranged from 0.1 to 0.7 mg kg^{-1} for the survival of adult *E. crypticus* and LC_{50} values that ranged from 0.2 to >1.0 mg kg^{-1} for the survival of adult *Enchytraeus albidus* in freshly amended SSL soil or in formulated soils with greater OM contents. However, the reproduction endpoints for *E. andrei* (cocoon production and viability, juvenile production) were more sensitive to CL-20 exposure in freshly amended SSL. This conclusion was based on EC_{50} values that ranged from 0.05 to 0.09 mg kg^{-1} (Robidoux et al., 2004) that were compared with our results for the soil invertebrate community. Similarly, reproduction toxicity benchmarks (EC_{50}) for enchytraeids and collembola, ranging from 0.08 to 0.7 mg kg^{-1} (Dodard et al., 2005; Kuperman et al., 2006a, 2006b), indicated greater toxicity of CL-20 compared with data from our microcosm assay. The greater established toxicity benchmark values (lower toxicity) for the survival of microarthropods and nematodes, compared with reproduction toxicity data for *E. fetida, E. andrei, E. crypticus, E. albidus,* and *F. candida* suggested that single-species toxicity tests may conservatively overestimate the exposure effects in natural soil ecosystems.

Table 3. Toxicity Benchmarks for Soil Invertebrates Established in Standardized Single-Species Toxicity Tests with CL-20-Amended SSL Soil

Species		LC_{50}	EC_{50}
Eisenia fetida		>500	0.1
	95% CI	ND	0.07–0.13
Enchytraeus crypticus		18	0.3
	95% CI	2.6–34	0.2–0.4
Folsomia candida		32	0.7
	95% CI	9–55	0.36–1.06

Notes: Toxicity benchmarks for mortality (LC_{50}) and reproduction (EC_{50}) endpoints were determined on the basis of concentration–response relationships in our previous studies (Kuperman et al., 2006a, 2006b) with earthworm *E. fetida* (ISO, 1998a), potworm *E. crypticus* (ISO 16387:2004), and collembolan *F. candida* (ISO, 1998b) using similar SSL soil freshly amended with CL-20. ND: not determined; no concentration–response relationships for mortality within tested concentration range.

4. CONCLUSIONS

This project was undertaken to investigate the effects of CL-20 on the soil microinvertebrate community, determine whether the toxicity of CL-20 to individual groups of the soil microinvertebrate community can be affected by the duration of exposure, and assess the utility of the microcosm assay as a tool for developing ecotoxicological parameters for use in ecological risk assessment (ERA) of contaminated soils. The results of our investigation, based on the number of taxonomic or functional (trophic) groups present in the individual treatments after 12 weeks, showed that the overall structure of the indigenous soil microarthropod or nematode communities in SSL soil was not affected by exposure to CL-20. However, further analysis of community structure revealed that predatory mesostigmatid mites and predatory nematodes had greater sensitivities to CL-20. The observed decreases in the populations of the respective predatory groups of the microinvertebrate community can result in the disruption of

the soil food web structure at impacted sites. Indigenous soil microarthropod and nematode communities exhibit distinctly different sensitivities to CL-20 in SSL soil. The total numbers of nematodes were either unaffected or increased after CL-20 treatments when compared with the carrier control. In contrast, CL-20 adversely affected the microarthropod community, as evidenced by significant decreases in the total numbers of microarthropods after CL-20 treatments when compared with the respective controls after 4 and 8 weeks of CL-20 exposure. This initial decrease was followed by ongoing recovery in the total numbers of microarthropods by the end of the 12 week exposure to CL-20, although the numbers continued to decline in a concentration-dependent manner.

The effects of CL-20 weathering-and-aging in soil upon the exposure of soil receptors were assessed through chronosequential harvesting of subset replicate units. This study was conducted to determine potential alterations in the bioavailability and resulting toxicity of CL-20 to microinvertebrates. The 12 week studies revealed a trend of decreasing toxicity of CL-20 over time for Collembola, prostigmatid mites, and predatory nematodes. But toxicity briefly increased for oribatid mites after 8 weeks before it returned to the approximate initial level after 12 weeks. These results showed the complexity of possible interactions among the physicochemical fate processes during weathering-and-aging of CL-20 in soil, changes in the bioavailability of the parent material and its possible degradation products, and the soil receptor-specific sensitivities of diverse groups from the soil invertebrate community.

Toxicity data for soil microarthropods and predatory nematodes were comparable with mortality data established in the standardized single-species toxicity tests with soil invertebrates exposed to CL-20 in a similar SSL soil. Greater estimated toxicity benchmark values (i.e., lower toxicity) for soil microarthropods, as well as abundance data for all nematode groups except predators, were compared with reproduction toxicity data established in the standardized single-species toxicity tests. These tests involved the earthworm *E. fetida*, potworm *E. crypticus*, and collembolan *F. candida* and suggest that single-species toxicity tests may conservatively overestimate the exposure effects in natural soil ecosystems. Overall, this investigation showed that community-level assessment and analysis of trophic structure of soil microfauna using a microcosm assay were sufficiently sensitive measures of chemical toxicity. In addition, these studies suggest that using a microcosm assay can bridge the gap between single-species toxicity tests and field studies. A microcosm assay can also be used to validate the ecotoxicity data from standardized laboratory tests and improve ERA by incorporating ecological principles into ERA methodologies.

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REFERENCES

ASTM International, Standard Specification for Reagent Water. In: *Book of ASTM Standards, Volume 11.01*; ASTM D1193-99; ASTM International: Philadelphia, PA, 2004; pp 116–118; UNCLASSIFIED Report; http://www.astm.org/DATABASE.CART/ HISTORICAL/D1193-99.htm (accessed February 2013).

Balakrishnan, V.K.; Halasz, A.; Hawari, J. Aqueous Hydrolysis of a New Energetic Compound, CL-20, under Alkaline Conditions. *Environ. Sci. Technol*. **2003**, *37*, 1838–1843.

Balakrishnan, V.K.; Monteil-Rivera, F.; Gautier, M.A.; Hawari, J. Sorption and Stability of the Polycyclic Nitramine Explosive CL-20 in Soil. *J. Environ. Qual*. **2004a**, *33*, 1362–1368.

Balakrishnan, V.K.; Monteil-Rivera, F.; Halasz, A.; Corbeanu, A.; Hawari, J. Decomposition of the Polycyclic Nitramine Explosive, CL-20, by Fe^0. *Environ. Sci. Technol*. **2004b**, *38*, 6861–6866.

Bogomolov, D.M.; Chen, S.-K.; Parmelee, R.W.; Subler, S.; Edwards, C.A. An Ecosystem Approach to Soil Toxicity Testing: A Study of Copper Contamination in Laboratory Microcosms. *Appl. Soil Ecol*. **1996**, *4*, 95–105.

Dodard, S.B.; Sunahara, G.I.; Kuperman, R.G.; Sarrazin, M.; Gong, P.; Ampleman, G.; Thiboutot, S.; Hawari, J. Survival and Reproduction of Enchytraeid Worms, Oligochaeta, in Different Soil Types Amended with Energetic Cyclic Nitramines. *Environ. Tox. Chem.* **2005**, *24*, 2579–2587.

Environment Canada (EC), *Guidance Document on Statistical Methods for Environmental Toxicity Tests*. EPS 1/RM/46; EC: Gatineau, Quebec, Canada. March 2005.

Edwards, C.A.; Bohlen, P.J. The Effects of Contaminants on the Structure and Function of Soil Communities. *Acta Zool. Fennica*. **1995**, *196*, 48–49.

International Organization for Standardization (ISO), *Soil Quality – Effects of Pollutants on Earthworms (*Eisenia fetida*) – Part 2: Determination of Effects on Reproduction*. ISO 11268-2:1998; Geneva, Switzerland, 1998a.

International Organization for Standardization (ISO), *Soil Quality: Effects of Pollutants on Enchytraeidae (*Enchytraeus *sp) – Determination of Effects on Reproduction and Survival*. ISO 16387:2004; Geneva, Switzerland, 2004.

International Organization for Standardization (ISO), *Soil Quality – Inhibition of Reproduction of Collembola* (Folsomia candida*) by Soil Pollutants*. ISO 11267:1998(E); Geneva, Switzerland, 1998b.

Kuperman, R.G.; Carreiro, M. Soil Heavy Metal Concentrations, Microbial Biomass and Enzyme Activities in a Contaminated Grassland Ecosystem. *Soil Biol. Biochem*. **1997**, *29*, 179–190.

Kuperman, R.G.; Checkai, R.T.; Garcia, M.V.; Römbke, J.; Stephenson, G.; Sousa, J.P. State of the Science and the Way Forward for the Ecotoxicological Assessment of Contaminated Land. *Brazilian Journal of Agricultural Research* (*Pesquisa Agropecuária Brasileira*, PAB) **2009**, *44*, 811–824.

Kuperman, R.G.; Checkai, R.T.; Simini, M.; Phillips, C.T.; Anthony, J.S.; Kolakowski, J.E.; Davis, E.A. Toxicity of Emerging Energetic Soil Contaminant CL-20 to Potworm *Enchytraeus crypticus* in Freshly Amended or Weathered and Aged Treatments. *Chemosphere* **2006a**, *62*, 1282–1293.

Kuperman, R.G.; Checkai, R.T.; Simini, M.; Phillips, C.T.; Anthony, J.S.; Haley, M.V.; Kolakowski, J.E.; Davis, E.A.; Chester, N.A.; Kurnas, C.W. *Environmental Fate and Transport of a New Energetic Material CL-20.* Strategic Environmental Research and Development Program (SERDP): Alexandria, VA; 2006b; http://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/ER-1254/ER-1254/(language)/eng-US (accessed February 2013).

Kuperman, R.G.; Checkai, R.T.; Simini, M.; Phillips, C.T.; Kolakowski, J.E.; Kurnas, C.W. Weathering and Aging of TNT in Soil Increases Toxicity to Potworm *Enchytraeus crypticus*. *Environ. Toxicol. Chem*. **2005**, *24*, 2509–2518.

Kuperman, R.G.; Knacker, T.; Checkai, R.; Edwards, C. Multispecies and Multiprocess Assays to Assess the Effects of Chemicals on Contaminated Sites. In *Environmental Analysis of Contaminated Sites*; Sunahara, G.; Renoux, A.; Thellen, C.; Gaudet, C.; Pilon, A., Eds.; John Wiley and Sons, Ltd.: Hoboken, NJ, 2002; pp 45–60.

Kuperman, R.G.; Phillips, C.T.; Checkai, R.T. Toxicity of Chemical Warfare Agent HD (Mustard) to the Soil Microinvertebrate Community in Natural Soils with Contrasting Properties. *Pedobiologia*, **2007**, *50*, 535–542.

Kuperman, R.G.; Williams, G.; Parmelee, R. Spatial Variability in the Soil Food Webs in a Contaminated Grassland Ecosystem. *Appl. Soil Ecol*. **1998**, *9*, 509–514.

McSorley, R.; Walter, D.E. Comparison of Soil Extraction Methods for Nematodes and Microarthropods. *Agric. Ecosyst. Environ*. **1991**, *34*, 201–207.

Merchant, V.A.; Crossley, D.E., Jr. An Inexpensive, High-Efficiency Tullgren Extractor for Soil Microarthropods. *J. Ga. Entomol. Soc*. **1970**, *5*, 83–87.

Monteil-Rivera, F.; Paquet, L.; Deschamps, S.; Balakrishnan, V.K.; Beaulieu, C.; Hawari, J. Physico-Chemical Measurements of CL-20 for Environmental Applications. Comparison with RDX and HMX. *J. Chromatogr*. *A.* **2004**, *1025*(1), 125–132.

Parmelee, R.W.; Phillips, C.T.; Checkai, R.T.; Bohlen, P.J. Determining the Effects of Pollutants on Soil Faunal Communities and Trophic Structure Using a Refined Microcosm System. *Environ. Toxicol. Chem*. **1997**, *16*, 1212–1217.

Parmelee, R.W.; Wentsel, R.S.; Phillips, C.T. Soil Microcosm for Testing the Effects of Chemical Pollutants on Soil Fauna Communities and Trophic Structure. *Environ. Toxicol. Chem*. **1993**, *12*, 1477–1486.

Robidoux, P.Y.; Sunahara, G.I.; Savard, K.; Berthelot, Y.; Dodard, S.; Martel, M.; Gong, P.; Hawari, J. Acute and Chronic Toxicity of the New Explosive CL-20 to the Earthworm (*Eisenia andrei*) Exposed to Amended Natural Soils. *Environ. Toxicol. Chem*. **2004**, *23*, 1026–1034.

Scott-Fordsmand, J.; Maraldo, K.; Van Den Brink, P. The Toxicity of Copper Contaminated Soil Using a Gnotobiotic Soil Multi-Species Test System (SMS). *Environ. Int.* **2008**, *34*, 524–530.

Stephenson, G.L.; Koper, N.; Atkinson, G.F.; Solomon, K.R.; Scroggins, R.P. Use of Nonlinear Regression Techniques for Describing Concentration–Response Relationships of Plant Species Exposed to Contaminated Site Soils. *Environ. Toxicol. Chem*. **2000**, *19*, 2968–2981.

Trott, S.; Nishino, S.F.; Hawari, J.; Spain, J.C. Biodegradation of the Nitramine Explosive CL-20. *Appl. Environ. Microbiol*. **2003**, *69*, 1871–1874.

U.S. Department of Agriculture, Natural Resources Conservation Service, Agricultural Research Service (USDA-NRCS/ARS). *Soil Taxonomy* (2nd ed.); Handbook No. 436; U.S. Government Printing Office: Washington, DC, 1999.

U.S. Environmental Protection Agency (USEPA). *Ecological Soil Screening Level Guidance*; OSWER 9285.7-55; USEPA: Washington, DC, 2005; http://www.epa.gov/ecotox/ecossl/pdf/ecossl_guidance_chapters.pdf (accessed February 2013).

U.S. Environmental Protection Agency (USEPA). Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC). Method 8330A, Revision 1, February, 2007. In: *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*. SW-846, 3rd ed.; Final Update IV; USEPA Office of Solid Waste: Washington, DC, 2007; http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/8330a.pdf (accessed February 2013).

Wentsel, R.S.; Beyer, W.N.; Edwards, C.A.; Kapustka, L.A.; Kuperman, R.G. Effects of Contaminants on Soil Ecosystem Structure and Function. In *Contaminated Soils: From Soil-Chemical Interactions to Ecosystem Management*; Lanno, R.L., Ed.; Society of Environmental Toxicology and Chemistry (SETAC) Press: Pensacola, FL, 2003; pp 117–159.

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ABBREVIATIONS AND ACRONYMS

ARS	Agricultural Research Service
ASTM	American Society for Testing and Materials
BDL	below detection limit
CAS	Chemical Abstracts Service
CI	confidence interval
CL-20	2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (China Lake compound 20)
CN	cyano
EC_{20}	effective concentration producing a 20% decrease in measurement endpoint
EC_{50}	effective concentration producing a 50% decrease in measurement endpoint
EC	Environment Canada
*EC*p	estimate of effect concentration for a specified percent effect
ERA	ecological risk assessment
FLSD	Fisher's least-significant difference
HPLC	high-performance liquid chromatography
ind g^{-1}	individuals per gram
ISO	International Organization for Standardization
LC	liquid chromatography
LC_{50}	median lethal concentration for 50% of the population
LOD	limit of detection
ND	not determined
NOEC	no-observed-effect concentration
NRCS	Natural Resources Conservation Service
OM	organic matter
p	probability value
PAR	photosynthetically active radiation
PTFE	polytetrafluoroethylene
r^2	regression coefficient
RH	relative humidity
RSD	relative standard deviation
SE	standard error
SERDP	Strategic Environmental Research and Development Program
SSL	Sassafras sandy loam
TNT	2,4,6-trinitrotoluene
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency


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